

ABSTRACT

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A method is provided for producing motif-specific, context-independent antibodies which are specific to at least one fixed amino acid residue, whether modified or unmodified, in the context of variable surrounding amino acid or peptide sequences. The method includes the steps of (1) constructing a peptide library featuring at least one fixed amino acid and variable surrounding amino acids, and (2) immunizing a host with this peptide library. Antibodies may optionally be isolated and purified from the antisera of this immunized host. The disclosed method encompasses both modified motifs, such as phosphothreonine, phosphoserine, MAPK substrate consensus sites, 14-3-3 consensus binding sites, CDK substrate consensus sites, PKA substrate consensus sites, Akt substrate consensus sites and acetylated lysine, as well as unmodified motifs.

Also provided are motif-specific, context-independent antibodies specific for at least one fixed amino acid in the context of variable surrounding peptide sequences, including phosphothreonine, phosphoserine, MAPK substrate consensus sites, 14-3-3 consensus binding sites, CDK substrate consensus sites, PKA substrate consensus sites, Akt substrate consensus sites and acetylated lysine, and other modified and unmodified amino acids as such fixed residues.

Methods for utilizing such antibodies to screen for unknown substrates of modification enzymes, enzymatic modification of substrates, including drug screens, and to identify enzymes which modify a given substrate are also provided.

Also provided are methods for profiling protein levels and post-translational modifications, including changes resulting from drug treatment, on a genomic scale by utilizing motif-specific, context-independent antibodies directed to conserved motifs on large and diverse protein populations.

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